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Enzymatic evidence for the operation of the FBP aldolase cleavage and TK/TA re-arrangement variant of the RuMP cycle in *Arthrobacter* P1

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1. INTRODUCTION

In the microbial assimilation of one-carbon compounds via the ribulose monophosphate (RuMP) pathway of formaldehyde fixation, cleavage of hexose phosphates to C₃ fragments and regeneration of the acceptor molecule RuMP are essential steps for the continued operation of the cycle. Two modes of cleavage and two modes of re-arrangement are known and the permutation of these leads to four possible variants of the RuMP cycle [1] (Table 1). The limited published data, reviewed by Zatman [2], suggest that so far only methylotrophic organisms with the KDPGA/TA

or the FBPA/SBPase combination have been encountered. In view of the fact that the KDPGA/SBPase variant is the energetically most unfavourable, it has been suggested [1,3] that organisms with this variant must be at a competitive disadvantage, which may explain why the occurrence of such organisms has not (yet) been reported. By the same reasoning, it must be considered surprising that unambiguous evidence for the exclusive operation in methylotrophs of the FBPA/TA variant, which has in fact the most favourable energy budget [3], has also not been published. The only indication for the possible occurrence of this variant comes from Strøm et al. [4] for *Pseudomonas methanica* and *Methylococcus capsulatus*, which have the TK/TA mode of re-arrangement and possess activities of both FBP aldolase and KDPG aldolase. However, in both organisms the other key enzyme of the FBP cleavage sequence, 6-phosphofructokinase, has such low specific activities, that this pathway can hardly be of physiological importance [2].

In this communication we wish to report enzymatic evidence for the exclusive operation of the energetically most favourable variant of the RuMP cycle in *Arthrobacter* P1. Our results suggest that this facultative methylotroph [5] uses the Embden–Meyerhof route for sugar phosphate cleavage and the TK/TA sequence of RuMP regeneration during growth on methylamine.

Table 1

Possible variants of the RuMP pathway of formaldehyde fixation

Variant no.	Mode of cleavage	Mode of re-arrangement
1	KDPGA	TK/TA
2	FBPA	TK/SBPase
3	KDPGA	TK/SBPase
4	FBPA	TK/TA

Abbreviations: KDPGA, 2-keto-3-deoxy-6-phosphogluconate aldolase; FBPA, fructose-1,6-bisphosphate aldolase; TK, transketolase; TA, transaldolase; SBPase, sedoheptulose-1,7-bisphosphatase.

2. MATERIALS AND METHODS

2.1. Organism

Arthrobacter P1 (NCIB11625) and its maintenance have been described previously [5].

2.2. Medium and cultivation

The organism was grown at 30°C in a stirred 2-l fermenter [6] containing 1 l of a mineral salts medium as described previously [5]. Carbon sources were added to the following final concentrations: methylammonium chloride, 45 mM; glucose, 10 mM; gluconate, 10 mM and acetate, 40 mM. During growth the pH was controlled at 7.0 by automatic adjustment with 1 N NaOH or 1 N H₂SO₄. Cells were harvested at the end of the exponential growth phase by centrifugation at 6000 × *g* for 10 min at 4°C, washed once with 50 mM potassium phosphate buffer pH 7.0 containing 5 mM MgSO₄ and resuspended in this buffer to a concentration of 10–20 mg dry weight/ml. These suspensions were either used directly for enzyme assays or stored at –20°C until required.

2.3. Assays of enzyme activity

Cell-free extracts were prepared as described by Levering et al. [5]. Spectrophotometric assays were performed with a Perkin–Elmer 124 spectrophotometer at 30°C and 340 nm, unless stated otherwise. In all assays the observed rate was linear for at least 2 min and proportional to the amount of extract added. Enzyme activities are expressed as munits/mg protein. One unit of activity is defined as that amount of enzyme catalyzing the transformation of 1 μmol of substrate or the formation of 1 μmol of product in 1 min.

2.3.1. Phosphoriboisomerase, EC 5.3.1.6

The reaction mixture (1 ml) contained: Tris–HCl buffer pH 7.6, 50 μmol; magnesium chloride, 5 μmol; thiamine pyrophosphate chloride, 0.2 μmol; α-glycerol phosphate dehydrogenase, 0.8 units; triose phosphate isomerase, 10 units; ribulose phosphate 3-epimerase, 0.2 units; TK, 0.1

units; NADH, 0.15 μmol; and extract. The reaction was started by the addition of 2 μmol ribose-5-phosphate.

2.3.2. Ribulose phosphate 3-epimerase, EC 5.1.3.1

The reaction mixture (1 ml) contained: Tris–HCl buffer pH 7.8, 50 μmol; magnesium chloride, 5 μmol; thiamine pyrophosphate chloride, 0.2 μmol; α-glycerol phosphate dehydrogenase, 0.8 units; triose phosphate isomerase, 10 units; phosphoriboisomerase, 1.75 units; TK, 0.1 units; NADH, 0.15 μmol; and extract. The reaction was started by the addition of 2 μmol ribose-5-phosphate.

2.3.3. Transketolase, EC 2.2.1.1

The reaction mixture (1 ml) contained: Tris–HCl buffer pH 7.8, 50 μmol; magnesium chloride, 5 μmol; thiamine pyrophosphate chloride, 0.2 μmol; α-glycerol phosphate dehydrogenase, 0.8 units; triose phosphate isomerase, 10 units; NADH, 0.15 μmol; and extract. The reaction was started by the simultaneous addition of 2 μmol ribose-5-phosphate and 0.4 μmol xylulose-5-phosphate.

2.3.4. Transaldolase, EC 2.2.1.2

The reaction mixture (1 ml) contained: triethanolamine–HCl buffer pH 8.0, 100 μmol; EDTA, 20 μmol; α-glycerol phosphate dehydrogenase, 0.8 units; triose phosphate isomerase, 10 units; NADH, 0.15 μmol; fructose-6-phosphate, 5 μmol; and extract. The reaction was started by the addition of 0.5 μmol erythrose-4-phosphate.

2.3.5. Sedoheptulose-1,7-bisphosphatase, EC 3.1.3.66

This enzyme was assayed discontinuously, using a P_i liberation method. The reaction mixture (0.8 ml) contained: Tris–maleate buffer pH 7.2, 50 μmol; magnesium chloride, 5 μmol; and extract. The reaction was started by the addition of 1 μmol sedoheptulose-1,7-bisphosphate and allowed to proceed for various periods of time (0–15 min), after which it was stopped by the addition of 0.2 ml 12% (w/v) trichloroacetic acid. Protein was removed by centrifugation and the supernatant was assayed for inorganic phosphate according to Chen et al. [7].

The following enzymes were assayed according to published procedures: 6-phosphogluconate dehydrogenase (EC 1.1.1.44), 6-phosphofructokinase (EC 2.7.1.11), FBPA (EC 4.1.2.13) and fructose-1,6-bisphosphatase (EC 3.1.3.11) according to Levering et al. [5]; combined activity of 6-phosphogluconate dehydratase (EC 4.2.1.12) and KDPGA (EC 4.1.2.14) according to Van Dijken and Quayle [8]; phosphoketolase (EC 4.1.2.9) according to Goldberg et al. [9].

2.4. Protein determinations

Protein was determined by the method of Lowry et al. [10], using bovine serum albumin as a standard.

2.5. Biochemicals and enzymes

Erythrose-4-phosphate, xylulose-5-phosphate, sedoheptulose-1,7-bisphosphate, ribulose phosphate 3-epimerase, phosphoriboisomerase and transketolase were obtained from Sigma Chemical

Co. (St. Louis, MO). All other biochemicals and enzymes were obtained from Boehringer, Mannheim, FRG.

3. RESULTS AND DISCUSSION

As reported previously [5], during growth on methylamine *Arthrobacter* P1 utilizes the RuMP cycle for the fixation of formaldehyde, which is formed by way of amine oxidase. A key enzyme of this carbon assimilation pathway, hexulose phosphate synthase, showed high activities in methylamine-grown cells, whereas during growth on glucose or acetate zero activity was found (Table 2). The failure to detect hexulose phosphate isomerase, the other key enzyme of this cycle, was discussed previously [5] and ascribed to an instability of this enzyme under the conditions used for the preparation of cell-free extracts and/or the spectrophotometric assay. After growth of *Arthrobacter* P1 in media containing methylamine, glucose or gluconate, the activities of 6-phos-

Table 2

Enzyme profiles of *Arthrobacter* P1 during growth on various substrates (activities are expressed as munits/mg protein). The numbers refer to enzymes indicated in Fig. 1

Enzyme	Growth substrate			
	Methylamine	Glucose	Gluconate	Acetate
(1) Hexulose phosphate synthase	742 ^a	0 ^a	—	0
(2) Hexulose phosphate isomerase	0 ^a	—	—	—
(3) 6-Phosphofructokinase	58 ^a	64 ^a	69	— (16 ^b)
(4) Fructose-1,6-bisphosphate aldolase	410 ^a	402 ^a	426	207 (145 ^b)
(5) Transketolase	836	225	346	169
(6) Transaldolase	1185	437	508	282
(7) Ribulose phosphate 3-epimerase	11300	604	824	522
(8) Phosphoriboisomerase 6-phosphogluconate dehydratase + KDPG aldolase	1105	218	318	197
6-Phosphogluconate dehydrogenase (NADP)	0	0	0	—
Sedoheptulose-1,7-bisphosphatase	238	291	736	160
Fructose-1,6-bisphosphatase	56	85	110	136
Phosphoketolase	21 ^a	30 ^a	55	70
	0	—	0	—

^a Data of Levering et al. [5].

^b Activity in ethylamine-grown cells [5].

0, not detectable.

—, not determined.

pho-fructokinase and FBPA in the cells were high as compared to those in cells grown on acetate or ethylamine. Under no conditions were activities of enzymes of the Entner–Doudoroff pathway detected (Table 2). This is unlike the situation in for instance *Escherichia coli* [11], which utilizes the FBP cleavage sequence during growth on glucose, but predominantly the Entner–Doudoroff route during growth on gluconate. In *Arthrobacter* P1 the levels of 6-phosphogluconate dehydrogenase and other enzymes of the pentose phosphate pathway were enhanced in gluconate-grown cells as compared to glucose-grown cells, while no activity of the xylulose-5-phosphate cleavage enzyme phosphoketolase was found (Table 2). This indicates that the metabolism of gluconate in *Arthrobacter* P1 is by way of the pentose phosphate pathway and, subsequently, the Embden–Meyerhof route, as reported for *Arthrobacter globiformis* and *Arthrobacter ureafaciens* [12]. It is therefore concluded that in *Arthrobacter* P1 cleavage of hexose phosphates into C_3 compounds only occurs via the Embden–Meyerhof route (Fig. 1).

The results of our studies on the mode of regeneration of RuMP as an acceptor molecule for formaldehyde fixation in *Arthrobacter* P1 suggest that this organism utilizes the TK/TA variant.

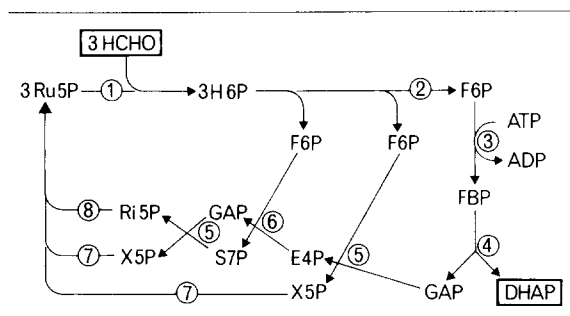


Fig. 1. RuMP pathway of formaldehyde fixation in *Arthrobacter* P1. The various reactions, indicated by numbers, are catalyzed by the corresponding enzymes listed in Table 2. Abbreviations: DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; E4P, erythrose-4-phosphate; Ru5P, ribulose-5-phosphate; Ri5P, ribose-5-phosphate; X5P, xylulose-5-phosphate; H6P, hexulose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; S7P, sedoheptulose-7-phosphate.

High activities of all four enzymes required for the conversion of two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate to three molecules of ribulose monophosphate, viz. transketolase, transaldolase, ribulose phosphate 3-epimerase and phosphoriboisomerase, were present in methylamine-grown cells. In contrast the activities of these enzymes were significantly lower in cells grown on non- C_1 substrates (Table 2). Activity of SBPase, which is a key enzyme of the TK/SBPase mode of rearrangement was also detected (Table 2), but methylamine-grown cells exhibited a significantly lower level of this enzyme than glucose-, gluconate- and acetate-grown cells. Since in addition the activity of this enzyme in methylamine-grown cells was only 5% of the transaldolase activity, it seems unlikely that SBPase plays any significant physiological role in the regeneration of RuMP during methylotrophic growth of *Arthrobacter* P1. It is of interest to note that the ratios of SBPase to FBPase activities in cell-free extracts of *Arthrobacter* P1 grown on various substrates are all of the same order of magnitude (Table 2), which suggests that one enzyme with dual specificity for both SBP and FBP might be operating. This is further indicated by the observation that the rate of dephosphorylation of FBP in extracts of *Arthrobacter* P1 was highest at a pH value of about 9.0, whereas dephosphorylation of SBP was optimal at neutral pH (see MATERIALS AND METHODS). A similar behaviour has been reported for the purified FBPase from rabbit liver [13].

In the present study convincing enzymatic evidence has been obtained for the operation of the FBPA/TA variant of the RuMP pathway as the sole route of carbon assimilation during growth of the facultative methylotroph *Arthrobacter* 2B2 (J.R. Quayle, personal communication) and it does not seem unreasonable to postulate that this energetically most favourable variant of the RuMP cycle may be more widespread amongst methylotrophs than is indicated by the data currently available (2).

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